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Origin of the Thiazole Ring of Camalexin, a Phytoalexin from *Arabidopsis thaliana*[1]

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The principal phytoalexin that accumulates in *Arabidopsis thaliana* after infection by fungi or bacteria is 3-thiazol-2′-yl-indole (camalexin). Detached noninoculated leaves of Arabidopsis and leaves inoculated with the fungus *Cochliobolus carbonum* were fed [^{35}S]cysteine (Cys) and [^{35}S]methionine. Inoculated leaves incorporated more than a 200-fold greater amount of radioactivity from [^{35}S]Cys into camalexin, as compared with noninoculated leaves. The amount of radioactivity from [^{35}S]Cys that was incorporated into camalexin from inoculated Arabidopsis leaves was 10-fold greater than the amount of radioactivity that was incorporated into camalexin from [^{35}S]methionine. Additional labeling experiments were performed to determine whether other atoms of Cys are incorporated into camalexin. [^{14}C]Cys and [^{35}S]Cys were incorporated into camalexin with approximately the same efficiency. Cys labeled either with deuterium (D_3-Cys[2,3,3]) or ^{13}C and ^{15}N ([U-^{13}C,^{15}N]Cys) was also fed to inoculated leaves of Arabidopsis; camalexin was analyzed by mass spectroscopic analysis. The average ratio of molecular ion intensities of 203/200 for [U-^{13}C,^{15}N]Cys-labeled camalexin was 4.22, as compared with 0.607 for the average 203/200 ratio for unlabeled camalexin. The mass fragment-ion intensity ratios of 60/58 (thiazole ring ion fragment) and 143/142 were also higher for [U-^{13}C,^{15}N]Cys-labeled camalexin, as compared with unlabeled camalexin. The 59/58 and 201/200 ratios were higher for D_3-Cys-labeled camalexin as compared with unlabeled camalexin. These data are consistent with the predicted formation of the thiazole ring of camalexin from Cys.

Many plant species produce antimicrobial compounds, known as phytoalexins, after pathogen infection. In general, members of a particular plant family will produce phytoalexins that belong to the same class of compounds (Bailey and Mansfield, 1982). Leguminous plants produce isoflavanoid and/or pterocarpan phytoalexins, whereas solanaceous plants produce sesquiterpenoid phytoalexins. All of the phytoalexins that have been characterized from the members of the Brassicaceae are indole derivatives that are substituted at the C-3 position with a sulfur-containing moiety (Hammerschmidt et al., 1993).

Conn et al. (1988) reported that *Camelina sativa* (false flax) accumulated two different phytoalexins after infection with *Alternaria brassicae*, a fungal pathogen of rapeseed. The most abundant of the two phytoalexins was identified as 3-thiazol-2′-yl-indole or camalexin (Browne et al., 1991). Camalexin is also the principal phytoalexin that accumulates in *Arabidopsis thaliana* (Tsuji et al., 1992). Anthranilate, but not Trp, was shown to be a biosynthetic precursor of camalexin through the use of the Trp-deficient mutants of Arabidopsis and feeding the detached leaves of Arabidopsis with radiolabeled anthranilate and Trp (Tsuji et al., 1993). Based on these results, we hypothesize that the camalexin biosynthetic pathway originates from an intermediate of the Trp pathway, which lies between anthranilate and indole.

A number of aldehydes possess the ability to react with Cys to form the corresponding thiazolidinecarboxylic acid (Greenstein and Winitz, 1961). It appears that either the sulfhydryl or amino group of Cys can add to the aldehyde followed by ring closure and the loss of water (Ratner and Clarke, 1937). Browne et al. (1991) first proposed a possible route of camalexin biosynthesis that involves the condensation of indole-3-carboxaldehyde with Cys followed by cyclization and decarboxylation. Based on this prediction of the biosynthesis of the thiazole ring of camalexin, we have formulated a detailed biosynthetic scheme (Fig. 1). According to this scheme, the formation of the thiazole ring of camalexin would involve two oxidation steps followed by a decarboxylation step starting from indole-3-thiazolidinecarboxylic acid. One of the reactants of this biosynthetic scheme, indole-3-carboxaldehyde, has been reported to accumulate in cabbage (*Brassica oleracea*; Devys and Barbier, 1991). The current study tested the hypothesis that the thiazole ring of camalexin originates from Cys.

MATERIALS AND METHODS

Plants (*Arabidopsis thaliana*, Columbia ecotype) were grown as described previously (Somerville and Orgren, 1982) in a growth chamber at 20°C for a 16-h light period and at 15°C for an 8-h dark period. Plants were grown under 100 μE $(m^2\ s)^{-1}$ (400–700 nm) of fluorescent illumination.

Synthesis of D_3-Cys and U-^{13}C,^{15}N-Cys

D_3-L-Ser (2,3,3) and [U-^{13}C,^{15}N]L-Ser were purchased from Cambridge Stable Isotopes (Andover, MA). ^{2}H-labeled Ser and U-^{13}C,^{15}N-labeled Ser were converted to the corresponding *O*-acetylserine derivative according to previously described methods (Kredich and Becker, 1971). Dry HCl was bubbled through 10 mL of glacial acetic acid until saturated. Saturation was determined by the presence

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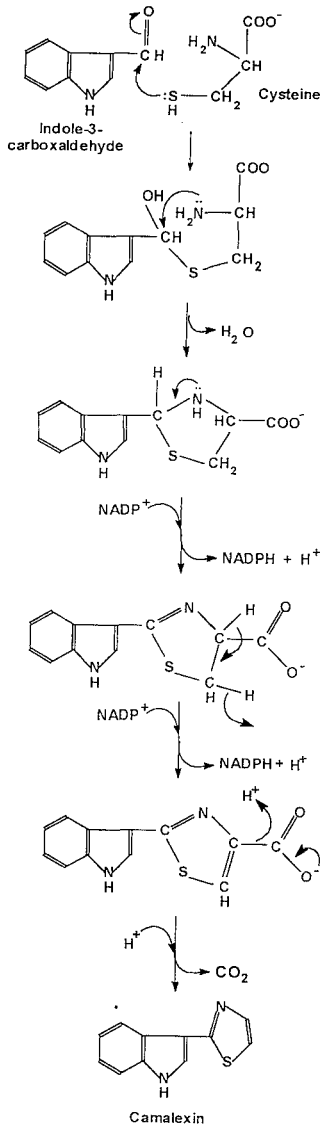

Figure 1. Hypothetical biosynthetic pathway for the thiazole ring of camalexin.

of chlorine in the gaseous effluent from the acetic acid solution, which was bubbled through an $AgNO_3$ solution. One hundred milligrams of labeled Ser was added to the solution, and the solution was stirred for 16 h at room temperature in a 50-mL screw-capped test tube. The solution was reduced to one-half volume under reduced pressure and then lyophilized. The identity of the synthesized product was checked by comparison with the authentic *O*-acetylserine using a 5-mercapto-2-nitrobenzoic acid assay of *O*-acetylserine sulfhydrylase activity (Tai et al., 1993). *O*-acetylserine sulfhydrylase from *Salmonella typhimurium* was kindly provided by Dr. Paul F. Cook (University of North Texas, Fort Worth).

To prepare the labeled Cys, 50 mL of 5 mM labeled *O*-acetylserine, 100 mM Hepes (pH 7.0), and 550 μg of *O*-acetylserine sulfhydrylase was divided equally into five 50-mL screw-capped test tubes. Twenty microliters of 100 mM Na_2S was added to each tube every 10 min during a 4-h period. The solution was filtered through a Centricon filter (Amicon, Beverly, MA; molecular weight cutoff, 10,000), acidified to pH 2.0 with 1.0 M HCl, and then applied to a 1.0- × 12-cm Dowex, Superdex 50X8-200 cation-exchange column (Sigma) pre-equilibrated with 0.1 M HCl. The column was rinsed with 50 mL of 0.1 M HCl and then with 50 mL of deionized H_2O. Labeled Cys was eluted from the column with 25 mL of 10 mM NH_4OH and the eluent was lyophilized.

Feeding of D_3-Cys and [U-^{13}C,^{15}N]Cys to Detached Leaves of Arabidopsis

Leaves were detached from 3- to 4-week-old plants using a razor blade. The detached leaves were then placed in a 15-cm-diameter Petri plate containing a premoistened 15-cm-diameter Whatman no. 1 filter paper. A suspension of *C. carbonum* (0.5 mL of 5×10^5 spores mL^{-1}) was placed on the underside of each leaf. After a 16-h incubation period at room temperature, the leaves were carefully blotted dry. A small segment was removed from the end of the petiole of each leaf under water using a razor blade. The leaf was then immediately placed in the tip of a 0.7-mL microcentrifuge tube containing 50 μL of 1 mg/mL labeled Cys in 5.0 mM β-mercaptoethanol. The concentration of labeled Cys was determined prior to the addition of β-mercaptoethanol (Kredich and Becker, 1971). Following uptake of the solution through the petiole, a fresh 50-μL aliquot of the Cys solution was added to the microfuge tube. After the second aliquot of the Cys solution had been taken up by the detached leaves, the leaves were placed upper surface down into 15-cm-diameter Petri plates with premoistened filter paper. Deionized H_2O (0.5 mL) was placed at the inoculation site on the lower surface of each leaf to replace the original spore inoculation droplet, and the leaves were incubated at room temperature for 24 h. Fifty leaves were used for each sample.

Feeding Radiolabeled Compounds to Detached Leaves of Arabidopsis

When different radiolabeled compounds were used in the same experiment, the specific activity of each compound was adjusted to the same value using the appropriate amount of the unlabeled compound. Each compound was fed to detached Arabidopsis leaves in a solution of 5.0 mM β-mercaptoethanol in a volume of 50 μL. After the solution had been taken up by the leaf, 50 μL of deionized H_2O was added to the microfuge tube. Deionized H_2O (0.5 mL) was placed at the inoculation site on the lower surface of each leaf to replace the original spore inoculation droplet, and the leaves were incubated at room temperature for 24 h. Ten leaves were used for each sample.

Extraction of Camalexin

Camalexin that is produced by Arabidopsis leaf tissue inoculated with *C. carbonum* will diffuse into the fungal spore inoculation droplet. Organic solvent extraction of

fungal spore inoculation droplets is a well-established technique for isolating phytoalexins (Bailey and Mansfield, 1982). Following the feeding with labeled compounds, detached leaves were allowed to incubate at room temperature in Petri plates for 24 h with a droplet of H_2O on the lower surface of the leaf. The H_2O droplet from each leaf that was used to replace the fungal spore inoculation droplet was removed, pooled, and extracted twice with an equal volume of hexane. The pooled hexane extracts were evaporated under a stream of N_2 and applied to a silica gel TLC plate. The hexane extract was fractionated by TLC using chloroform:methanol (9:1, v/v) as the solvent. The location of camalexin on the TLC plate following development with the TLC solvent was visualized under long-wave UV illumination. The camalexin spot was then scraped into a scintillation vial for counting of radioactivity. For mass spectroscopic analysis and HPLC quantitation, camalexin was eluted from the silica gel with ethyl acetate using a sintered glass funnel. The ethyl acetate was then evaporated under a stream of N_2.

Quantitation of Camalexin

Following evaporation of the ethyl acetate, the remaining residue was redissolved in 100 μL of hexane:isopropanol (93:7, v/v) just prior to HPLC analysis. The HPLC mobile phase (hexane:isopropanol [93:7, v/v]) was pumped through a 5-μm 150- × 4.6-mm silica column (Econosphere, Alltech, Deerfield, IL) at a flow rate of 1.0 mL/min. The eluant from the column was monitored at 210 nm using a variable wavelength detector. The amount of camalexin in each sample was determined by a comparison to the HPLC detector response of injections of known amounts of the camalexin standard.

RESULTS

To determine the possible origin of the sulfur atom in the thiazole ring of camalexin, detached, noninoculated leaves of Arabidopsis and leaves inoculated with the maize pathogen *C. carbonum* were fed [^{35}S]Cys and [^{35}S]Met (Table I). Inoculation of Arabidopsis leaves with *C. carbonum* elicited camalexin accumulation (Fig. 2). A comparison was made between these two labeled amino acids in terms of the efficiency of the incorporation of radioactivity into camalexin. Labeled Ser was included in this study because Ser is a precursor of Cys via *O*-acetylserine in plants, and labeled anthranilic acid and Trp were included in the experiment to confirm the results of our previous work (Tsuji et al., 1993). There was a large increase in the incorporation of radioactivity from either Cys or anthranilate into camalexin from inoculated leaves, as compared with noninoculated leaves. The magnitude of this difference was much smaller when labeled Ser, Trp, or Met was fed to detached leaves. The incorporation of radioactivity into camalexin from [^{35}S]Cys was more than 10-fold greater than the incorporation of radioactivity from [^{35}S]Met or [^{3}H]Ser for Arabidopsis leaves inoculated with *C. carbonum*. Likewise, the specific activity of labeled camalexin was more than 5-fold greater when [^{35}S]Cys was used as the source of radioactivity, as compared with [^{35}S]Met. The incorporation of radioactivity into camalexin from [^{14}C]anthranilate was more than 20-fold greater than the incorporation of radioactivity from [^{3}H]Trp for leaves inoculated with *C. carbonum*. The specific activity of labeled camalexin from inoculated leaves was also 20-fold greater when [^{14}C]-anthranilate was used as the source of radioactivity, as compared with [^{3}H]Trp.

The only compound present in the hexane extracts of water droplet diffusates from the fungus-inoculated leaves of Arabidopsis, which incorporated label from either anthranilate or Cys, appears to be camalexin (Fig. 3). The observation that camalexin is present in undetectable levels in water droplet diffusates from noninoculated leaves of Arabidopsis (data are not shown) and that there is a low level of radioactivity that "incorporated" into camalexin in the noninoculated controls (Table I; Fig. 3) suggests that there is a very low level of incorporation of label from Cys or anthranilate into compounds other than camalexin that will partition into hexane from water droplet diffusates from fungus-inoculated or noninoculated leaves.

The data presented in Table I and Figure 3 provide strong evidence that the sulfur atom of the thiazole ring of

Table I. *Incorporation of labeled Ser, Trp, anthranilate, Met, and Cys into camalexin from inoculated and noninoculated leaves of A. thaliana*

Label	Treatment	Radioactivity Incorporated	Specific Activity
		dpm[a]	*dpm/μg camalexin*
[^{3}H]Ser	Control	493 ± 225	–[b]
[^{3}H]Ser	Elicited	357 ± 70	186 ± 81
[^{3}H]Trp	Control	123	–
[^{3}H]Trp	Elicited	550 ± 86	339 ± 38
[^{14}C]Anthranilate	Control	770 ± 396	–
[^{14}C]Anthranilate	Elicited	14,886 ± 5,732	8880 ± 3320
[^{35}S]Met	Control	124 ± 8	–
[^{35}S]Met	Elicited	1,540 ± 292	860 ± 116
[^{35}S]Cys	Control	60 ± 20	–
[^{35}S]Cys	Elicited	21,846 ± 1,634	4990 ± 140

[a] The specific activity of each labeled compound was adjusted to 25 mCi/mmol. To each sample 2.47 × 10^6 dpm of [^{3}H(G)]Ser, 2.55 × 10^6 dpm of 5-[^{3}H]Trp, 1.83 × 10^6 dpm of [ring-^{14}C(U)]anthranilate, 4.05 × 10^6 dpm of [^{35}S]Met, and 4.35 × 10^6 dpm of [^{35}S]Cys were applied. The data are means ± SE of two samples. [b] –, Not determined.

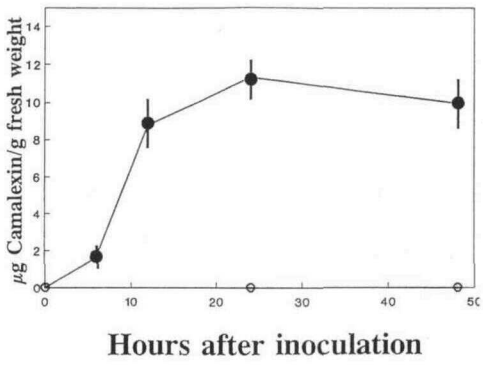

Figure 2. Accumulation of camalexin in noninoculated (○) or *C. carbonum*-inoculated Arabidopsis leaves (●). The *C. carbonum* spore concentration used for inoculation was 5×10^5 spores/mL. Five leaves were used per treatment. The error bars represent the SD of three determinations.

camalexin originates from Cys. In our attempt to determine whether part of the carbon backbone of Cys is also incorporated into camalexin, [^{14}C]Cys was fed to noninoculated and inoculated leaves of Arabidopsis (Table II). High levels of incorporation of [^{35}S]Cys and [^{14}C]Cys into camalexin were observed. These results suggest that both the sulfur and carbon atoms from Cys are incorporated into the thiazole ring of camalexin.

To obtain direct evidence that a portion of the Cys molecule is incorporated intact into camalexin, an attempt was made to label Cys with stable isotopes for MS analysis. Although commercially available, Cys labeled uniformly with ^{13}C and ^{15}N or with ^{2}H atoms, replacing the hydrogen atoms of the α and side-chain carbon atoms, is prohibitively expensive. Cys labeled in the desired atoms, however, can be synthesized from labeled Ser from a combination of both chemical and enzymatic steps (Kredich and Becker, 1971).

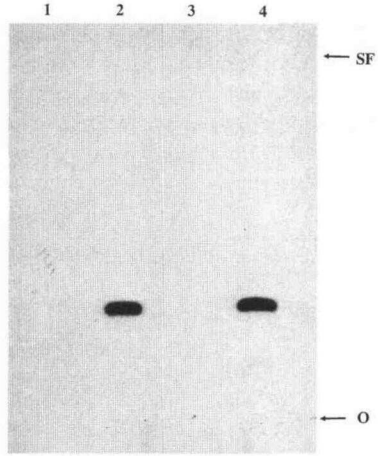

Figure 3. Autoradiogram of a TLC separation of hexane extracts of H_2O droplets placed on noninoculated Arabidopsis leaves (lanes 1 and 3) or *C. carbonum*-inoculated Arabidopsis leaves (lanes 2 and 4) following the feeding of radioactivity to the leaves from either [^{14}C]anthranilate (lanes 1 and 2) or [^{35}S]Cys (lanes 3 and 4) as described in "Materials and Methods." The amount of radioactivity and specific activity of [^{14}C]anthranilate and [^{35}S]Cys was the same as described in Table I. The locations of the origin (O) and solvent front (SF) of the TLC plate are indicated by arrows. The TLC solvent was chloroform:methanol (99:1, v/v).

Table II. *Incorporation of [^{35}S]Cys and [^{14}C]Cys into camalexin*

Label	Treatment	Radioactivity Incorporated	Percentage of Incorporation
		dpm[a]	
[^{35}S]Cys	Control	126 ± 44	0.003 ± 0.001
[^{35}S]Cys	Elicited	13,352 ± 18	0.282 ± 0.001
[^{14}C]Cys	Control	56 ± 16	0.008 ± 0.002
[^{14}C]Cys	Elicited	3,021 ± 41	0.417 ± 0.007

[a] The specific activity of each labeled compound was adjusted to 300 mCi/mmol. To each sample (10 detached leaves) 4.73×10^6 dpm of [^{35}S]Cys and 7.25×10^5 dpm of [^{14}C]cystine were applied. Each labeled compound was fed to detached leaves of Arabidopsis in 5 mM β-mercaptoethanol, which readily reduces the amount of [^{14}C]cystine fed to detached leaves to [^{14}C]Cys (25-fold molar excess of β-mercaptoethanol over [^{14}C]cystine). The data are means ± SE of two samples.

Cys labeled with either ^{2}H or ^{13}C and ^{15}N was fed through petioles of *C. carbonum*-inoculated leaves of Arabidopsis. Labeled camalexin was then extracted as previously described and analyzed by mass spectroscopy (Table III; Fig. 4). If the proposed biosynthesis of the thiazole ring shown in Figure 1 is correct, then labeling with [U-^{13}C,^{15}N]Cys should increase the mass of camalexin by 3 mass units to 203. To normalize for differences in the absolute value of mass fragment-ion intensities between different labeled camalexin samples, the data shown in Table III are in terms of the ratio of the ion intensity of the expected mass ion of labeled camalexin (or mass fragment)/the ion intensity of the mass ion of unlabeled camalexin (or mass fragment). The mean value from three determinations for the ratio of the ion intensity of the expected mass ion of [U-^{13}C,^{15}N]Cys-labeled camalexin (203)/the ion intensity of the mass ion of unlabeled camalexin (200) for camalexin labeled with [U-^{13}C,^{15}N]Cys is 4.22. The same mean value for the ratio of the ion intensity of 203/ion intensity of 200 for unlabeled camalexin is 0.607. This result is consistent with the hypothetical biosynthetic scheme shown in Figure 1 and suggests a level of label enrichment of 3 to 4%. The mass fragment-ion intensity ratios of 60/58 and 143/142 are also higher for [U-^{13}C,^{15}N]Cys-labeled camalexin, as compared with unlabeled camalexin. This result is also consistent with the theorized pattern of labeling (Fig. 4). Ratios of mass fragment-ion intensities for camalexin labeled with D_3-Cys showed no difference from unlabeled camalexin in the 143/142 ratios, as expected, but there was also no difference in the 60/58 ratio. If two atoms of ^{2}H atoms from D_3-Cys had incorporated into camalexin, then the 60/58 ratio would be greater for D_3-Cys-labeled camalexin, as compared with unlabeled camalexin. However, there was a difference in the 59/58 ratio. The 201/200 ratio was also higher for D_3-Cys-labeled camalexin, as compared with unlabeled camalexin. This result suggests that only one ^{2}H atom from D_3-Cys is incorporated into the thiazole ring of camalexin.

Table III. *Ratio of mass fragment intensities from camalexin labeled with [U-$^{13}C_3$,^{15}N]Cys, D_3-Cys, or unlabeled camalexin*

Label	Ratio of Mass Fragment-Ion Intensities (×100)[a]					
	59/58	60/58	143/142	201/200	202/200	203/200
Unlabeled	4.97[a]	5.28[a]	18.6[a]	14.9[a]	5.72[a]	0.607[a]
[U-$^{13}C_3$,^{15}N]Cys	5.62[a]	7.44[b]	22.6[b]	16.1[b]	6.46[b]	4.22[b]
D_3-Cys	9.08[b]	5.41[a]	17.6[a]	18.9[c]	7.05[b]	1.43[a]

[a] Different letter superscripts represent significant differences of means of three determinations for each column (Student's *t*-test [$P \leq 0.05$]).

DISCUSSION

Results from the current study provide strong evidence that Cys forms the thiazole ring of camalexin in Arabidopsis, thus supporting the initial hypothesis (Browne et al., 1991; Tsuji et al., 1992). The sulfur atom in the thiazole ring was initially shown to originate from Cys using [^{35}S]Cys. Inoculation of Arabidopsis leaves with the nonpathogenic fungus *C. carbonum* greatly increased the incorporation of radioactivity from [^{35}S]Cys into camalexin, as compared with noninoculated controls. This result would be expected if Cys were a precursor of camalexin synthesis, since inoculation of Arabidopsis leaves with *C. carbonum* elicits the accumulation of camalexin (Fig. 2). The incorporation of radioactivity into camalexin from anthranilate, a known precursor of camalexin synthesis (Tsuji et al., 1993), also increases after inoculation with *C. carbonum*. Evidence that two of the carbon atoms and the nitrogen atom of the thiazole ring of camalexin are also derived from Cys comes from [^{14}C]cystine- and [$^{13}C_3$,^{15}N]Cys-labeling experiments. Label from [^{14}C]cystine incorporated into camalexin at about the same efficiency as label from [^{35}S]Cys. This result suggests that, along with the sulfur atom, at least one carbon atom of the thiazole ring of camalexin comes from Cys. [$^{13}C_3$,^{15}N]Cys labeling, in combination with mass spectroscopic analysis, provides more direct evidence of the thiazole ring formation from Cys.

The ratio of intensities of mass ion fragments for the molecular ion (203/200) and fragmentation of the thiazole ring (60/58 and 143/142) are consistent with the formation of the thiazole ring from Cys. The mass spectroscopic analysis of camalexin labeled with D_3-Cys suggests that two hydrogen atoms are lost from the side chain of Cys during the formation of the thiazole ring. It is expected that at least one hydrogen atom originating from the side chain of Cys would be lost during a dehydrogenation step, but it is also possible that another hydrogen atom is lost during this dehydrogenation step just prior to decarboxylation (Fig. 1). Unfortunately, the low level of enrichment of camalexin from D_3-labeled Cys (approximately 3%) prevented a determination of the position of the incorporated ^{2}H atom in the thiazole ring of camalexin by NMR analysis.

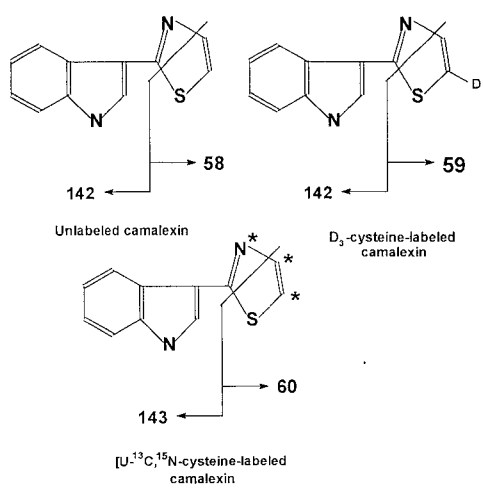

Figure 4. Principal mass fragments of unlabeled camalexin and putative principal mass fragments of camalexin labeled with [U-$^{13}C_3$,^{15}N]Cys or D_3-Cys. Asterisks indicate expected position of ^{15}N and ^{13}C atoms in camalexin from [U-^{13}C,^{15}N]Cys.

At present there is no direct evidence for indole-3-carboxaldehyde as a biosynthetic precursor of camalexin in Arabidopsis. It is equally plausible that indole-3-carboxylic acid rather than indole-3-carboxaldehyde reacts with Cys to eventually form camalexin. The amino or sulfhydryl group of Cys could add to the carboxyl group of indole-3-carboxylic acid to form an amide or thioester bond, respectively. In this alternate biosynthetic scheme, the next step would be ring closure followed by a rearrangement and the loss of water, resulting in the formation of indole-3-thiazolinecarboxylic acid. The last two steps in camalexin synthesis would then be the same as shown in Figure 1.

Indole glucosinolates, produced by some members of the Brassicaceae, are structurally similar to the crucifer phytoalexins. Because of these structural similarities, it has been proposed that indole glucosinolates may serve as biosynthetic intermediates for some crucifer phytoalexins (Monde et al., 1994). Although camalexin shares some structural similarities to other crucifer phytoalexins, the thiazole group of camalexin is structurally unrelated to the crucifer phytoalexins that contain a methylthiol group, such as brassinin. Also, it is not known how a thiazole group could arise from methyl isothiocyanate, as proposed for the biosynthesis of brassinin (Monde et al., 1994). The data from the current and previous reports (Tsuji et al., 1993) do not support the hypothesis that indole glucosinolates are precursors of camalexin, since Trp, the biosynthetic precursor of indole glucosinolates, is at best a poor precursor of camalexin.

To our knowledge, this report provides the first evidence of a thiazole ring formation originating from Cys in plants. There is precedence for thiazole ring formation from Cys in bacteria (Favret et al., 1992). Microcin B17 is a member of a group of low-molecular-weight polypeptide antibiotics produced by many different species of enteric bacteria (Genilloud et al., 1989). Microcin B17 undergoes posttranslational modification that involves the backbone of the

peptide and leads to the formation of four thiazole and four oxazole rings, which originate from four Cys and four Ser residues (Yorgey et al., 1994). The biosynthesis of a structurally related metabolite thiamine, which contains a thiazole ring with an attached methyl group, is thought to occur by a more complicated pathway than the biosynthetic scheme shown in Figure 1. Several different compounds contribute to the formation of the thiazole ring of thiamine in plants (Julliard and Douce, 1991). Apparently, the camalexin biosynthetic pathway is different from the thiamine pathway, since Arabidopsis thiamine biosynthetic mutants are not deficient in camalexin production (J. Glazebrook, personal communication).

The elucidation of the thiazole ring formation of camalexin is an important step toward determining the biosynthetic steps involved in camalexin synthesis. The relatively few biosynthetic steps required for the formation of the thiazole ring of camalexin from Cys (Fig. 1) may simplify the characterization of *pad* mutants (Glazebrook and Ausubel, 1994). Knowledge of the biosynthetic steps likely to be involved in the formation of the thiazole ring of camalexin will also aid in the identification and characterization of enzymes that catalyze these thiazole-forming reactions. Together with the knowledge of the camalexin pathway, both the genetic and biochemical manipulation of Arabidopsis will further the study of the signal transduction pathway that leads to the induction of camalexin biosynthesis.

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